THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH

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NEW YORK 21, N. Y.

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Dear Josh:

Thanks for the kind words but I'm some put out by you. You put me in a rather awkward position with Bruce. I think that you should have consulted with me prior to putting the question of authorship to him. Besides I disagree with you both as to Z and L authorship.

As Bruce stated in his letter to me all of his results were based on experiments which I had well under way when he arrived and the only reason they were given up was my leaving and his subsequent progress along these lines. I also feel that your progeny tests are necessary for the completion of the manuscript. It seems silly to me not to include them or to publish them separately when they are available and had you not done them I should have as would Bruce had he had a phage for PLT-22 resistant lines. (I've just sent him LP*-30 and 36).

Otherwise I think the outline is complete. Some caution should be used however in statements as to non-allelism of F- genes. When working with non-isogenic stocks(between strains or species) one can't be certain that because two strains can transduce each other the primary genes concerned are not alleles. Of course the larger the checkorboards the less likelyhood of error, but still one could be merely labeling suppressors. Even progeny testing can't be of much help here.

The lytic variant is from PLT-22/2 and selected on LT-2. The plaques are very clear on S. gal and B 1411. On LT-7 and Lt-2 they are muddy but easily differentiated from parent. The mutant gives total recovery of infective centers at multiplicities to five with survivorship as expected on a Poisson. With parent phage 57 % survivors at one (lysogenics sensitives and mottled) and 85% above three. Would be a nice system to study lysogenicity as by using S. gal as an indicator surviving colonies can be counted directly on soft agar plate due to the more rapid growth of typhimurium. Thus with parent phage find colonies central to plaques not with mutant phage.

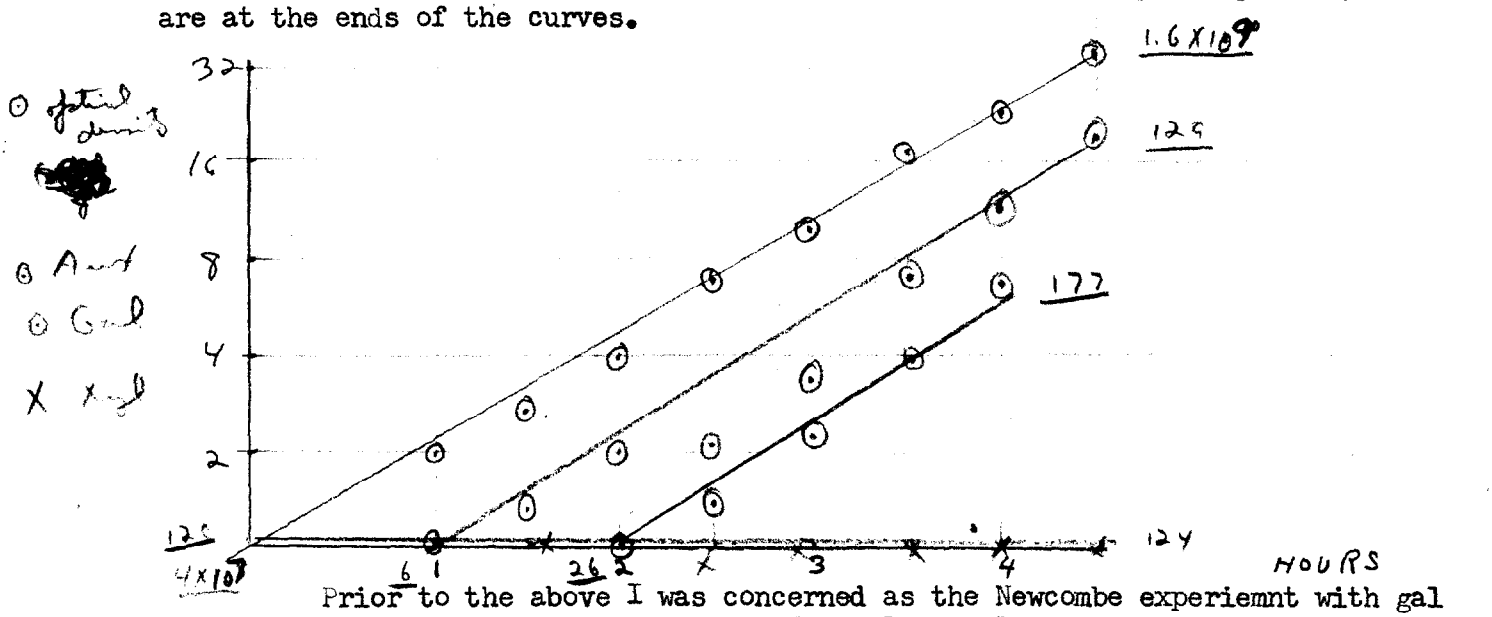
The serum k was about 80 / minute.

Your U.V data are some different than mine. With your lamp six minutes gave a return to unit survivorship, of FA as tested with SW-435 Xyl Gal and Aux. Activation ratio was respectively 6-4-3. How were your irradiations accomplished and at what multiplicities did you assay (phage active or inactive)? Have you run a complete phage inactivation curve, mine appeared to be two-hit.

Another FA* phage correlation. The more phage adsorbed the more transductions. Thus 191 and 665 which can adsorb at least 10 times more phage than LT-22 can be transduced with maximum frequency of one in 50,000. The particles seem independent up to saturation.

By playing with the conditions I have been able to Lwoff LT-22 with a yield of 3 X 10^8 / ml. It has transducing activity comparable to a LT-2 lysate of the same titer. Also tried thiomalate but as you know Salmonella just thrive on reducing conditions, no phage was detected. This latter method probably completely an idiosyncrasy of megaterium.

I have so far had little luck in getting dense populations to turn over in broth even with slow feeding but am still trying. However did the delay experiment in another manner. SW-351 was given 20 minutes at 37 C to adsorb 1.5 PLT-22/547 particles (used this phage as I knew that at least for gal it had a lower phage to FA ratio). No bacterial growth could occur at this density, 5 X 10^9/ ml. One tenth of this was plated on some dozen prewarmed NA plates and incubated. At intervals the growth was washed off two plates per point and plated for Xyl Gal and Aux transinductions. Sample was also read in nephelometer. I recovered about 4/5 of the plating sample after allowing it to dry in on control plates. Percentagewise this should get better as the density increases. Also 4/5 of the transinductions were recovered as compared to the direct plating of the adsorption mixture. The results are plotted below on a ln 2 scale. The initial and final numbers per 0.1 growth plate are at the ends of the curves.



Prior to the above I was concerned as the Newcombe experiemnt with gal had given a maximum of 4-5 generations for gal stabilization while a track often had many more micro-colnies. This apparently differs for each character. The order is also interesting Xyl Gal Aux . With three of anything you get in a sense a position test and gal is always in the middle;faster stabilization, lower spontaneous mutation rate, lower frequency of transduction, lower U.V. activation, ? as to U.V resistance. I have a feeling that this is more than a coincidence perhaps a reflection of particle size.

The mouse-virulence problem continues to go well. Perhaps too well as it takes more and more of my time. Shouldn't say that as it really is getting interesting. (just gave a staff meeting here and hope to now be able to hit up the director for a technician). We now know that you can switch the mice to a natural diet on the day the "immunizing" dose is given and still get the diet affect -- again helpful in the substances resolution. The data on the spleen counts of virulent organisms two days after the seondary challenge with the virulents are not homogenous (natural diet),

in fact obviously bimodal although here my knowledge of statistics fails me -- how do ypu prove thid ? Anyway the numbers of the mice clustered at the two apparent means are in accord with the numbers that live or die on this diet -- 15% die the rest survive. 10-20% have [illegible] at the high mean the rest at the low. The decision between life or death is apparently made alsmot immediatley after challenge. The data on the synthetic diet are not as clear but also not homogeneous, here 65% deaths expected. We can test the homogeneity of our sampling procedures by mice tha have received only virulent organsims and are sacrificed two days later. These data are homogeneous.

In broth the virulent and avirulent grow equally well and stay in the same ratio in mixed culture. How to mimic the two day prior challenge in a test tube I don't know. But a mouse is a mouse etc.

Best regards

Sincerely,

Norton

P.S If you ever have a chance would love to hear what else has been going on in the lab.